

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)	PATENT APPLICATION
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Linda G. Lee, et al.)	Group Art Unit: Not Yet Assigned
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Application Serial No.: Not Yet Assigned)	Examiner: Not Yet Assigned
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Filed: Herewith)	
)	
Title: ENERGY TRANSFER DYES WITH)	
<u>ENHANCED FLUORESCENCE</u>)	

PRELIMINARY AMENDMENT

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

Applicants submit this Preliminary Amendment concurrently with the above-referenced application.

Enclosed are new Figures 4A, 4B and 8 and redlined copies of Figures 4A, 4B and 8. Also enclosed herewith are formal drawings for the application.

Also enclosed is a Sequence Listing in paper and computer readable form, as well as a Statement under 37 CFR 1.821(f) that the submitted Sequence Listing in paper and computer readable form are the same.

Please amend the application as follows.

In the Drawings

Please replace Figures 4A, 4B and 8 with enclosed new Figures 4A, 4B and 8. Redlines of Figures 4A, 4B and 8 are provided showing the changes that have been made.

In the Specification

Please replace the paragraph beginning at line 8, on page 1 with the following rewritten paragraph:

This application is a continuation of U.S. Application Serial No. 09/272,097, filed on March 18, 1999, which is a continuation of U.S. Application Serial No. 09/046,203, filed on March 23, 1998, now Patent No. 5,945,526, which is a continuation of U.S. Application No. 08/726,462, filed on October 4, 1996, now Patent No. 5,800,996, which is a continuation-in-part of U.S. Application No. 08/672,196, filed on June 27, 1996, now U.S. Patent No. 5,847,162 which is a continuation-in-part of U.S. Application No. 08/642,330, filed on May 3, 1996, now Patent No. 5,863,727, each of which it is incorporated herein by reference in its entirety.

Please replace the paragraph beginning at line 20, on page 10, with the following rewritten paragraph:

In another embodiment, the energy transfer fluorescent dyes have donor and acceptor dyes with the general structure where Y_1 and Y_2 taken separately are either hydroxyl, oxygen, iminium or amine, the iminium and amine preferably being a tertiary iminium or amine and R_{11} - R_{16} are any substituents which are compatible with the energy transfer dyes of the present invention.

Please replace all of page 52 with the following rewritten paragraphs:

In compound 3A-A, one of R_1 and R_2 is ethyl, the other being hydrogen, R_3 and R_4 taken separately are hydrogen, R_5 is methyl, R_6 and R_7 - R_{10} taken separately are hydrogen, X_1 is carboxylate, and one of X_3 and X_4 is a linking group, the other being hydrogen.

In compound 3A-B, one of R_1 and R_2 is ethyl, the other being hydrogen, R_3 and R_4 taken separately are methyl, R_5 is methyl, R_6 - R_{10} taken separately are hydrogen, X_1 is carboxylate, and, one of X_3 and X_4 is a linking group, the other being hydrogen.

In compound 3A-C, R₁ and R₂ taken separately are methyl, R₃ and R₉ taken together form a six membered ring, R₄ and R₈ taken together form a six membered ring, R₅, R₆, R₇, and R₁₀ taken separately are hydrogen, X₁ is carboxylate, and, one of X₃ and X₄ is a linking group, the other being hydrogen.

In compound 3B-D, R₁ and R₂ taken separately are hydrogen, R₃ and R₉ taken together form a six membered ring, R₄ and R₈ taken together form a six membered ring, R₅, R₆, R₇, and R₁₀ taken separately are hydrogen, X₁ is carboxylate, and one of X₃ and X₄ is a linking group, the other being hydrogen.

In compound 3B-E, one of R₁ and R₂ is ethyl, the other being hydrogen, R₃ and R₉ taken together form a six membered ring, R₄ and R₈ taken together form a six membered ring, R₅ is methyl, R₆, R₇ and R₁₀ taken separately are hydrogen, X₁ is carboxylate, and, one of X₃ and X₄ is a linking group, the other being hydrogen.

In compound 3B-F, R₁ and R₂ taken separately are hydrogen, R₃ and R₄ taken separately are methyl, R₅-R₁₀ taken separately are hydrogen, X₁ is carboxylate, and, one of X₃ and X₄ is linking group, the other being hydrogen.

Please replace the paragraph beginning at line 5, on page 53 with the following rewritten paragraph:

Figure 4A shows a generalized synthesis wherein the substituent X₁ can be other than carboxylate. In the figure, X' indicates moieties which are precursors to X₁. In the method illustrated in Figure 4A, two equivalents of a 3-aminophenol derivative 4A-A/4A-B, such as 3-dimethylaminophenol, is reacted with one equivalent of a dichlorobenzene derivative 4A-C, e.g., 4-carboxy-3,6-dichloro-2-sulfobenzoic acid cyclic anhydride, i.e., where the X₁' moieties of 4c taken together are,

Please replace the paragraphs beginning at line 14, on page 53 with the following rewritten paragraphs:

The reactants are then heated for 12 h in a strong acid, e.g., polyphosphoric acid or sulfuric acid, at 180°C. The crude dye 4A-D is precipitated by addition to water and isolated

by centrifugation. To form a symmetrical product, the substituents of reactants 4A-A and 4b are the same, while to form an asymmetrical product, the substituents are different.

Figure 4B shows a generalized synthesis wherein the substituent X_1 is carboxylate. In the method of Figure 4B, two equivalents of a 3-aminophenol derivative 4A-A/4A-B, such as 3-dimethylaminophenol, is reacted with one equivalent of a phthalic anhydride derivative 4B-E, e.g. 3,6-dichlorotrimellitic acid anhydride. The reactants are then heated for 12 h in a strong acid, e.g., polyphosphoric acid or sulfuric acid, at 180°C. The crude dye 4A-D is precipitated by addition to water and isolated by centrifugation. To form a symmetrical product, the substituents of reactants 4A-A and 4A-B are the same, while to form an asymmetrical product, the substituents are different.

Please replace the paragraph beginning at line 23, page 93, with the following rewritten paragraph:

Dye primer sequencing was performed on the pGEM (SEQ. ID. NO.: 3) using a set of four dyes attached to the M13-21 primer (SEQ. ID. NO.: 2) as described in Example 5. Figure 13 is a four color plot of the dye labeled oligonucleotides produced from the sequencing. The peak for cytosine corresponds to the fluorescence of 5-CFB-DR110-2. The peak for adenosine corresponds to the fluorescence of 6-CFB-DR6g-2. The peak for guanosine corresponds to the fluorescence of 5-CFB-DTMR-2. The peak for thymidine corresponds to the fluorescence of 5-CFB-DROX-2.

Please replace pages 95-98 of the Specification regarding the Sequence Listing with the attached replacement pages 95-98.

In the Claims:

Please delete claims 2-79.

REMARKS

Attached hereto is a marked-up version of the changes made to the specification, sequence listing and claims by the current amendment. The attached marked-up version is captioned "**Version with markings to show changes made.**"

Should the Examiner have any questions, the Examiner is encouraged to telephone the undersigned.

Respectfully submitted,

Date: Oct. 29, 2001

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The paragraph beginning at line 8, page 1 has been amended as follows:

[This application is a continuation-in-part of "ENERGY TRANSFER DYES WITH ENHANCED FLUORESCENCE,," Application Serial No.: 08/642,330; Filed: May 3, 1996 and U.S. Application Serial No.: 08/672,196; filed June 27, 1996; entitled: "4,7-DICHLORORHODAMINE DYES" which are incorporated herein by reference.]

This application is a continuation of U.S. Application Serial No. 09/272,097, filed on March 18, 1999, which is a continuation of U.S. Application Serial No. 09/046,203, filed on March 23, 1998, now Patent No. 5,945,526, which is a continuation of U.S. Application No. 08/726,462, filed on October 4, 1996, now Patent No. 5,800,996, which is a continuation-in-part of U.S. Application No. 08/672,196, filed on June 27, 1996, now U.S. Patent No. 5,847,162 which is a continuation-in-part of U.S. Application No. 08/642,330, filed on May 3, 1996, now Patent No. 5,863,727, each of which it is incorporated herein by reference in its entirety.

The paragraph beginning at line 20, on page 10, has been amended as follows:

In another embodiment, the energy transfer fluorescent dyes have donor and acceptor dyes with the general structure where Y_1 and Y_2 taken separately are either hydroxyl, oxygen, iminium or amine, the iminium and amine preferably being a tertiary iminium or amine and $[R_{11}-R_{17}]$ $R_{11}-R_{16}$ are any substituents which are compatible with the energy transfer dyes of the present invention.

Page 52 has been amended as follows:

In compound [3a] 3A-A, one of R_1 and R_2 is ethyl, the other being hydrogen, R_3 and R_4 taken separately are hydrogen, $[R_5]$ R_6 is methyl, $[R_6-R_{10}]$ R_5 and R_7-R_{10} taken separately

are hydrogen, X_1 is carboxylate, and one of X_3 and X_4 is a linking group, the other being hydrogen.

In compound [3b] 3A-B, one of R_1 and R_2 is ethyl, the other being hydrogen, R_3 and R_4 taken separately are methyl, R_5 is methyl, R_6 - R_{10} taken separately are hydrogen, X_1 is carboxylate, and, one of X_3 and X_4 is a linking group, the other being hydrogen.

In compound [3c] 3A-C, R_1 and R_2 taken separately are methyl, R_3 and $[R_7] R_2$ taken together form a six membered ring, R_4 and R_8 taken together form a six membered ring, R_5 , R_6 , $[R_9] R_7$, and R_{10} taken separately are hydrogen, X_1 is carboxylate, and, one of X_3 and X_4 is a linking group, the other being hydrogen.

In compound [3d] 3B-D, R_1 and R_2 taken separately are hydrogen, R_3 and $[R_7] R_2$ taken together form a six membered ring, R_4 and R_8 taken together form a six membered ring, R_5 , R_6 , $[R_9] R_7$ and R_{10} taken separately are hydrogen, X_1 is carboxylate, and one of X_3 and X_4 is a linking group, the other being hydrogen.

In compound [3e] 3B-E, one of R_1 and R_2 is ethyl, the other being hydrogen, R_3 and $[R_7] R_2$ taken together form a six membered ring, R_4 and R_8 taken together form a six membered ring, R_5 is methyl, R_6 , $[R_9] R_7$ and R_{10} taken separately are hydrogen, X_1 is carboxylate, and, one of X_3 and X_4 is a linking group, the other being hydrogen.

In compound [3f] 3B-F, R_1 and R_2 taken separately are hydrogen, R_3 and R_4 taken separately are methyl, R_5 - R_{10} taken separately are hydrogen, X_1 is carboxylate, and, one of X_3 and X_4 is linking group, the other being hydrogen.

The paragraph beginning at line 5, on page 53 has been amended as follows:

Figure 4A shows a generalized synthesis wherein the substituent X_1 can be other than carboxylate. In the figure, X' indicates moieties which are precursors to X_1 . In the method illustrated in Figure 4A, two equivalents of a 3-aminophenol derivative [4a/4b] 4A-A/4A-B, such as 3-dimethylaminophenol, is reacted with one equivalent of a dichlorobenzene derivative [4c] 4A-C, e.g., 4-carboxy-3,6-dichloro-2-sulfo benzoic acid cyclic anhydride, i.e., where the X_1' moieties of 4c taken together are,

The paragraphs beginning at line14, on page 53, have been amended as follows:

The reactants are then heated for 12 h in a strong acid, e.g., polyphosphoric acid or sulfuric acid, at 180°C. The crude dye [4d] 4A-D is precipitated by addition to water and isolated by centrifugation. To form a symmetrical product, the substituents of reactants [4a] 4A-A and 4b are the same, while to form an asymmetrical product, the substituents are different.

Figure 4B shows a generalized synthesis wherein the substituent X_1 is carboxylate. In the method of Figure 4B, two equivalents of a 3-aminophenol derivative [4a/4b] 4A-A/4A-B, such as 3-dimethylaminophenol, is reacted with one equivalent of a phthalic anhydride derivative [4e] 4B-E, e.g. 3,6-dichlorotrimellitic acid anhydride. The reactants are then heated for 12 h in a strong acid, e.g., polyphosphoric acid or sulfuric acid, at 180°C. The crude dye [4d] 4A-D is precipitated by addition to water and isolated by centrifugation. To form a symmetrical product, the substituents of reactants [4a] 4A-A and [4b] 4A-B are the same, while to form an asymmetrical product, the substituents are different.

The paragraph beginning at line 23, page 93, has been amended as follows:

Dye primer sequencing was performed on the [M13 (SEQ. ID. NO.: 2)] pGEM (SEQ. ID. NO.: 3) using a set of four dyes attached to the M13-21 primer (SEQ. ID. [NO. 3] NO.: 2) as described in Example 5. Figure 13 is a four color plot of the dye labeled oligonucleotides produced from the sequencing. The peak for cytosine corresponds to the fluorescence of 5-CFB-DR110-2. The peak for adenosine corresponds to the fluorescence of 6-CFB-DR6g-2. The peak for guanosine corresponds to the fluorescence of 5-CFB-DTMR-2. The peak for thymidine corresponds to the fluorescence of 5-CFB-DROX-2.